unknown structure that also form asymmetric homodimers, indicating that both subunits can assume open and closed conformations. The linkage between conformational and binding equilibria is a key factor in the assembly of complex biomolecular systems such as RT.
Comparison of hydrogen/deuterium exchange patterns indicates that the secondary structure of the polymerase domains of p66 and p51 monomers is almost identical, but that both p66 and p51 are less structured as free monomers than they are in the heterodimer. Small angle X-ray scattering (SAXS) was employed to study the conformations of the monomers in solution. Scattering curves were not well fit by monomeric structures derived from the crystal structure of the heterodimer. Better fits were obtained using rigid body modeling and allowing the subdomains to rotate with respect to each other to optimize fits to the scattering data. Multiple optimizations yielded ensembles of 500 structures per monomer. The p51 clusters could be grouped in 2 clusters of closely related conformations; the p66 structures showed greater variability with 20 conformational clusters. The 2 clusters for p51 resemble the closed and open conformations seen in the crystal structure. The 20 clusters of p66 comprise a variety of open and closed conformations. This conformational heterogeneity is consistent with the conformational selection mechanism proposed to explain the slow subunit binding and inhibitor binding kinetics.

234-Pos Board B20
The Many Conformations of Epac2: A Cyclic-AMP Sensing Cellular Regulator Studied Via Solution X-Ray Scattering (SAXS) & Hydrogen Deuterium Exchange Mass Spectrometry
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Epac2 is a guanine nucleotide exchange factor which is directly activated by cAMP. According to the model of Epac activation, a localized ''hinge'' motion is a major change in the Epac structure upon cAMP binding. in this study, we test the functional importance of hinge bending for Epac activation by targeted mutagenesis. We show that substitution of the conserved residue phenylalanine 435 by glycine facilitates the hinge bending, and is constitutively active, while tryptophan, impedes the hinge motion and results in a dramatic decrease in Epac2 catalytic activity. Structural parameters for wild type Epac and two of its mutants determined by small-angle X-ray scattering (SAXS) further confirm the importance of hinge motion in Epac activation. in addition, peptide amide hydrogen/deuterium exchange mass spectrometry (DXMS) was used to probe the solution structural and conformational dynamics of full length Epac2. Our study also suggests that the side-chain size of the amino acid at the position 435 is a key to Epac functioning. It seems that phenylalanine at this position has the optimal size to prevent ''hinge'' bending and keep Epac closed and inactive in the absence of cAMP while still allowing the proper hinge motion for full Epac activation in the presence of cAMP. The DXMS results also support this mechanism in which cAMP-induced Epac2 activation is mediated by a major hinge motion centered on the C-terminus of the second cAMP binding domain. These results suggest that in addition to relieving the steric hindrance imposed upon the catalytic lobe by the regulatory lobe, cAMP may also be an allosteric modulator directly affecting the interaction between Epac2 and RAP1.

Protein Dynamics I

235-Pos Board B21
Single-Molecule Fluorescence Spectroscopy of the Structure and Dynamics of the Spliceosomal Complex
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The spliceosome is the cellular machinery responsible for removing non-coding introns from precursor mRNA. During its catalytic action the spliceosome undergoes compositional and conformational changes. We are investigating the conditions for recruitment and release of particular proteins during the splicing steps. We determine how the changes occur (stepwise or in a correlated manner) and the roles of certain spliceosomal RNA helicases in the restructuring of the complex. The spectroscopic methods we use for investigating the spliceosomal complex are Dual-Focus Fluorescence Correlation Spectroscopy (2fFCS) and Dual-Color-Fluorescence Cross-Correlation Spectroscopy (2-color-FCCS). These methods allow for studying structural and dynamical properties of proteins and small nuclear ribonucleoproteins (snRNPs). 2-color-FCCS in combination with 2fFCS enables the observation of protein-protein interactions and the determination of dissociation constants for protein-protein and protein-mRNA bindings which could not be resolved with standard biochemical methods.
First, we focus on the B to Bact transition followed by the LSm ring proteins LSm4 and LSm7. With 2-color-FCCS and 2fFCS, we determine how both proteins are released from the complex and their molecular size by measuring their diffusion coefficients.
Second, we observed the thermally-stable splicing factor Cwc25. We could determine, under which conditions it binds to the complex, when it is released and the conditions for stable binding of Cwc25 to the spliceosome. By measuring several mutants we could answer the question whether Cwc25 is released before or during the second catalytic step.
Third, we focused on the proteins of the snRNP U2 complex. It is a highly debated question, when the U2 subunit is released from the complex and whether the U2 proteins are released at different stages of the splice cycle. We monitored the sequential release of different U2 proteins (Prp21, Cus1) during the first and second catalytic step.

236-Pos Board B22
Dynamics of Mitochondria in Adult Rat Cardiomyocytes
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It is becoming clear that the structure of the cell is important for the organization and energy metabolism of cells. Previous studies in our lab showed how mitochondria in adult cardiomyocytes are arranged regularly within Intracellular Energetic Units in a longitudinal lattice at the level of A-band between the myofibrils and located within the limits of the sarcomers (Biochim Biophys Acta, 2010, 1797: 678-97). in the present study the mitochondrial position and dynamics were studied in adult rat cardiomyocytes in which Z-lines were labelled by fluorescent α-actinin. Fast scanning of intact adult cardiomyocytes by transfected GFP-α-actinin was carried out using a line scanning LSM7 LIVE and LSM710 confocal microscope. The results show that cytoskeletal proteins position each mitochondrion in a specific area and make fusion and fission physically unfeasible processes. The fast and simultaneous line scanning in two fluorescent channels synchronized with the piezo stage axial displacements allow rapid 3D imaging of the mitochondrial dynamics referenced to the structural elements of sarcomers. The on-going research work aims at capturing dynamic behaviour of both intra- and extra-mitochondrial regulatory proteins in correlative manner.
This study was performed in the framework of ANR project SYBECAR, France.

237-Pos Board B23
Measuring Actin Flow in Cell Protrusion in 3D
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Cells generate forces through the cytoskeleton that are transmitted to the extracellular matrix via cell adhesion in order to adhere and migrate. Though cellular force generation has been studied in detail in 2D environment, less is known about cytoskeleton dynamics of cells embedded in natural 3D matrices.
Fluorescent Speckle Microscopy (FSM) has been used to capture high-resolution images of actin turnover dynamics within living cells in 2D. However, this method is not applicable when cells are in 3D environment due to lower resolution and signal level.
In this work we developed a new method to capture actin flow in 3D with high spatial-temporal resolution. Modulation tracking and correlation spectroscopy techniques were combined to show the directional flow of actin in 3D live cell. MDA-MB 231 cells with actin-GFP expression were cultured in type I collagen. The laser bin was oscillating back and forth at nearby two cross sections on cell protrusion, while scanning in orbit manner at each plane. The orbit radius was modulated in order to detect actin flow in the center of the protrusion. The intensity carpets from two positions were then cross-correlated.
We found that actin in cell protrusion in 3D is relatively stable. Most of the measurement showed no correlation of two positions while some showed directional flow near protrusion surface. The actin flow rate of cell protrusion in 3D measured using this method is in the order of seconds, which is comparable to 2D results using FSM.
With this method, we are able to visualize and quantify actin flow in 3D without the need of special speckle vectors and with high spatial-temporal resolution.
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